Volume 9 Article 44

4-1-1982

Research Notes : Genetic linkage analysis

T. E. Devine
Cell Culture and Nitrogen Fixation Laboratory

B. H. Breithaupt
Cell Culture and Nitrogen Fixation Laboratory

CELL CULTURE AND NITROGEN FIXATION LABORATORY
BELTSVILLE AGRICULTURAL RESEARCH CENTER
Beltsville, Maryland 20705

1) Genetic linkage analysis

The Rj_2 gene (Caldwell, 1966), which conditions ineffective nodulation with strains of the c1 and 122 serogroup of *Rhizobium japonicum* (Kirchner) Buhanan, and the Rj_4 gene (Vest and Caldwell, 1972), conditioning an ineffective nodulation response with rhizobial strain 61 of the Beltsville Culture Collection *R. japonicum*, were tested for linkage association with the gene L_1 (black pod). The rj_1 gene (Williams and Lynch, 1954), conditioning a non-nodulating response with a broad spectrum of strains of *R. japonicum*, was tested for linkage with the gene *fr* (roots nonfluorescent in UV light) and L_1 (black pod). The gene y_9 (chlorophyll defective) was tested for possible linkage with the genes *ln* (narrow leaf), and P_1 (glabrous). The P_1 gene was also tested for linkage with the gene *fr*.

Materials and Methods. Genetic stocks (T lines) and Clark rj_1 rj_1 were obtained from the Soybean Genetic Type Collection (Bernard and Weiss, 1973). Crosses were made in the field (Table 1) and F_1 seed were advanced to the F_2 generation in the greenhouse. In some crosses, the F_2 progeny were assayed directly for expression of the pertinent phenotype. In other crosses, the F_3 progeny were assayed and the F_2 genotype rationalized from the F_3 phenotypes. F_3 seed was produced in the field at Beltsville. Crosses with the Rj_2 and Rj_4 genes were evaluated in plastic growth tray assemblies (Devine and Reisinger, 1978) and inoculated with 7-day-old broth cultures of strain 7 of *R. japonicum* from the Beltsville Culture Collection to define for Rj_2 and strain 61 to define for Rj_4.

The progeny of crosses T135 x T109 and T135 x T145 were evaluated for chlorosis, narrow leaf and glabrous traits in the F_2 generation in the field at Beltsville. In the case of the T215 x 'Clark' rj_1 cross, each F_3 seed lot was divided between two packets and two 10-foot-row plantings were made in the field. The first planting was dug and the F_3 plants were scored for nodulation response to determine the presence of the rj_1 gene. The field had been used for soybean cultivation for many years previously and contained abundant *R. japonicum*. The second planting was allowed to mature and the progeny rows were scored for segregation of the black pod trait.

In the cross T145 x 'Minsoy', F_2 seed were germinated in petri dishes and examined, 3 days after the beginning of water imbibition, under UV light for fluorescence of the radical. After classification for fluorescence, the seedlings were transplanted into rows in growth trays, cultured in a growth room, and classified for the glabrous character at the first trifolioliate-leaf stage. For the cross of Clark rj_1 x Minsoy, F_2 seedlings were classified for fluorescence as previously described, then were inoculated with strain 7 of *R. japonicum* and transplanted into rows in vermiculite-filled growth trays. After an additional 3 weeks growth, the seedlings were removed from the vermiculite and classified for nodulation response.

In the cross T215 x 'Hardee', each F_3 seed lot was divided and about 50 seed were evaluated for the presence of the nodulation response gene Rj_2 under conditions of controlled inoculation with rhizobial strain 7 in growth trays in the greenhouse. The remaining seed was planted in 10-foot rows in

the field at Beltsville, MD, and grown to maturity when rows were classified for segregation for the black pod trait. Similarly, in the 'Hill' x T215 cross, the F_3 seed lots were divided and one portion of seed was characterized for the black pod trait in the field and the other portion was characterized for the Rj_4 nodulation response gene in growth trays after inoculation with rhizobial strain 61.

Results. Results of the seven linkage tests are given in Table 2. All of the traits tested displayed independent assortment indicating they were not genetically linked to the genes tested.

Table 1.

Cross	Generation of progeny evaluated	Trait characterized		
		In field	In growth trays	In petri dishes
T135 (y_9 *LN*) x T109 (Y_9 *ln*)	F_2	y_9 *ln*	--	--
T135 ($y_9 p_1$) x T124 ($Y_9 P_1$)	F_2	y_9 p_1	--	--
T215 (L_1 Rj_1) x Clark rj_1 ($l_1 rj_1$)	F_3	l_1 rj_1	--	--
T145 (*Fr* P_1) x Minsoy (*fr* p_1)	F_2	--	p_1	*fr*
Clark rj_1 (rj_1 *Fr*) x Minsoy (Rj_1 *fr*)	F_2	--	rj_1	*fr*
T215 (L_1 rj_2) x Hardee (l_1 Rj_2)	F_3	L_1	rj_2	--
Hill (Rj_4 l_1) x T215 (rj_4 L_1)	F_3	L_1	rj_4	--

Table 2. Soybean genetic linkage tests

Genes	a	b	c	d	Sum	%R*	SE	Phase
	T135 (y_9 y_9 *Ln Ln*) x T109 (Y_9 Y_9 *ln ln*)							
Y_9 y_9 *Ln ln*	171	45	52	14	282	50	4	R
	T215 (L_1 L_1 rj_2 rj_2) x Hardee (l_1 l_1 Rj_2 Rj_2)							
L_1 l_1 Rj_2 rj_2	55	11	20	6	92	55	7	R
	Hill (Rj_4 Rj_4 l_1 l_1) x T215 (rj_4 rj_4 L_1 L_1)							
Rj_4 rj_4 L_1 l_1	132	34	27	5	198	45	6	R
	T135 (y_9 y_9 p_1 p_1) x T145 (Y_9 Y_9 P_1 P_1)							
Y_9 y_9 P_1 p_1	144	40	55	17	256	49	5	C
	T145 (P_1 P_1 *Fr Fr*) x Minsoy (p_1 p_1 *fr fr*)							
P_1 p_1 *Fr fr*	291	91	102	32	516	50	3	C
	Clark rj_1 (rj_1 rj_1 *Fr Fr*) x Minsoy (Rj_1 Rj_1 *fr fr*)							
Rj_1 rj_1 *Fr fr*	76	31	24	10	141	50	6	R
	T215 (L_1 L_1 Rj_1 Rj_1) x Clark rj_1 (l_1 l_1 rj_1 rj_1)							
L_1 l_1 Rj_1 rj_1	115	31	44	14	204	48	5	R

*Recombination percentages calculated by the product method (Immer and Henderson, 1943).

References

Bernard, R. L. and M. G. Weiss. 1973. Qualitative genetics, pp. 117-154. *In*: B. E. Caldwell (ed.) Soybeans: Improvement, production and uses. American Society of Agronomy, Madison, WI.

Caldwell, B. E. 1966. Inheritance of a strain-specific ineffective nodulation in soybean. Crop Sci. 6:427-428.

Devine, T. E. and W. W. Reisinger. 1978. A technique for evaluating nodulation response of soybean genotypes. Agron. J. 70:510-511.

Immer, F. R. and M. T. Henderson. 1943. Linkage studies in barley. Genetics 28:419-440.

Vest, Grant and B. E. Caldwell. 1972. Rj_4 -- a gene conditioning ineffective nodulation in soybeans. Crop Sci. 12:692-694.

Williams, L. F. and D. L. Lynch. 1954. Inheritance of a non-nodulating character in the soybean. Agron. J. 46:28-29.

T. E. Devine
B. H. Breithaupt